one wildtype tyrosine residue is replaced with an amino acid that is not capable of being phosphorylated.

REMARKS

Reconsideration of the present application in view of the present amendment and the following remarks is respectfully requested. Claims 1-33 are currently pending. Claims 17-33 are withdrawn as being directed to a non-elected invention, and claim 14 is withdrawn as being directed to a non-elected species. Applicants hereby cancel claims 2, 6 and 7 without prejudice to the filing of any related divisional, continuation, or continuation-in-part application. Claim 1 has been amended to more clearly define the subject matter encompassed by Applicants' invention, and claim 8 has been amended solely to correct what would be an otherwise improper dependency in view of the cancellation of claim 6. Support for the amended claims may be found in the specification, for example, at page 2, line 26 through page 4, line 7; page 15, line 13 through page 21, line 12; page 28, line 14 through page 31, line 6. In the specification, the paragraphs beginning at page 2, line 26; at page 15, line 13; and at page 16, line 4 have been amended to correct an inadvertent typographical error in the sequence identifier number (SEQ ID NO) of the protein tyrosine phosphatase (PTP) signature motif and to render the specification consistent with the Sequence Listing. As described in the specification, for example, at page 15, line 14, and as disclosed in the Sequence Listing, the SEQ ID NO for the PTP signature motif [I/V]HCXAGXXR[S/T]G is SEQ ID NO:1. No new subject matter has been added.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version With Markings to Show Changes Made**."

OBJECTIONS TO THE CLAIMS

The PTO objects to claims 9, 10, 13, and 16. Specifically, the PTO asserts that the claims are directed to non-elected species.

Applicants respectfully submit that in response to the requirement for an election of species regarding claims 9-16, an election has been made in Paper No. 12 as acknowledged by the PTO (Action, at page 2). Applicants submit further that according to 37 C.F.R. §1.141, upon

allowance of a generic claim, claims directed to additional species which are written in dependent form or that otherwise include all the limitations of an allowed generic claim are entitled to consideration. Insofar as the instant claims depend directly or indirectly from a generic claim, Applicants wish to hold in abeyance any requirement that claims 9, 10, 13 and 16 be amended to recite a single disclosed species, until such time as the scope of the allowable generic claim is confirmed.

REJECTION UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, WRITTEN DESCRIPTION

The PTO rejects claims 1-13 and 15-16 under 35 U.S.C. § 112, first paragraph, asserting that the claimed subject matter lacks adequate written description in the specification. In particular, the PTO alleges that claims 1-13 and 15-16 are directed to a genus of mutant protein tyrosine phosphatases (PTPs), and that claims 1-4, 6-13, and 15-16 are directed to a genus of substrates that bind to the mutant PTP. The PTO further asserts that the claimed genera are highly variant and that the specification teaches the structures of only a few representative species of PTP1B mutants and substrates that bind to the mutants. In addition, the PTO alleges that the specification fails to describe any other representative species by any identifying characteristic or property other than function.

Applicants respectfully traverse this rejection and submit that, as disclosed in the specification and recited in the pending claims, Applicants possessed the claimed invention at the time the application was filed. Applicants' invention is directed in pertinent part to a method for identifying an agent which alters the interaction between a protein tyrosine phosphatase (PTP) and a tyrosine phosphorylated polypeptide which is a substrate of the PTP, comprising (a) contacting in the absence and in the presence of a candidate agent, a substrate trapping mutant of a PTP and a detectably labeled tyrosine phosphorylated peptide which is a substrate of the PTP under conditions and for a time sufficient to permit formation of a complex between the tyrosine phosphorylated peptide and the substrate trapping mutant PTP, wherein the substrate is capable of generating a fluorescence energy signal and wherein the substrate trapping mutant protein tyrosine phosphatase is selected from the group consisting of (i) a protein tyrosine phosphatase in which wildtype protein tyrosine phosphatase catalytic domain invariant aspartate residue is replaced with an amino acid which does not cause significant alteration of the Km of the enzyme

but which results in a reduction in Kcat to less than 1 per minute, and (ii) a protein tyrosine phosphatase in which a cysteine that is present in a signature sequence motif as set forth in SEQ ID NO:1 within a wildtype protein tyrosine phosphatase catalytic domain is mutated at an amino acid position occupied by a cysteine residue; and (b) comparing the fluorescence energy signal level in the absence of the agent to the fluorescence energy signal level in the presence of the agent, wherein a difference in the fluorescence energy signal level indicates the agent alters formation of a complex between the PTP and the substrate, and wherein the fluorescence energy signal is a fluorescence polarization signal.

Applicants submit that the specification reasonably describes what is claimed by providing sufficiently detailed, relevant, and identifying characteristics of (i) PTP substrate trapping mutants, and (ii) tyrosine phosphorylated polypeptide PTP substrates, for use in the claimed method. Contrary to the assertion by the PTO, Applicants submit that the specification provides explicit guidance describing the correlation between the structure of PTP substrate trapping mutants and the function of these mutants.

The present application extensively describes structures as well as functions of PTPs from which substrate trapping mutant PTPs may be derived, for example, at page 1, line 25 through page 2, line 3; page 2, line 26 through page 4, line 7; page 15, lines 13-19; page 16, line 10 through page 17, line 27; page 20, lines 13-22; (including publications cited in these cited passages, see page 11, lines 11-13); and in Figure 1A-1E and the Sequence Listing. Applicants are therefore somewhat puzzled by the PTO's assertion of a lack of representative species, where a plethora of PTP sequences are provided along with a clear description that Applicants possessed the wherewithal to prepare trapping mutants from them, and to identify suitable substrates for them (*e.g.*, U.S. Pat. Nos. 5,912,138 and 5,951,979; U.S.A.N. 09/323,426; all incorporated by reference in the present specification, as are Sun *et al.* (MKP), 1993 *Cell* 75:487; Flint *et al.* (PTP1B), 1997 *Proc. Nat. Acad. Sci.* 94:1680; Garton *et al.* (PTP-PEST), 1996 *J. Mol. Cell. Biol.* 16:6408; Tiganis *et al.* (TCPTP), 1998 *Mol. Cell Biol.* 18:1622; Spencer *et al.* (PTP-HSCF), 1997 *J. Cell Biol.* 138:845; Zhang *et al.* (PTPH1), 1999 *J. Biol. Chem.* 274:17806).

Additionally, the specification describes substrate trapping mutant PTPs in which the PTP invariant aspartate residue is replaced with an amino acid that does not cause significant

alteration of the Km of the enzyme but which results in a reduction in Kcat to less than 1 per minute (less than 1 min^{-1}) *(e.g.*, page 18, lines 13-17). Optionally, a substrate trapping mutant PTP may further comprise other mutations, such as replacement of a catalytic domain cysteine residue, and/or at least one tyrosine residue replaced with an amino acid that is not capable of being phosphorylated (*Id.*). Therefore, the specification teaches which amino acids in a PTP polypeptide sequence (such as those abundantly disclosed in the specification and drawings, *e.g.*, Fig. 1) may be altered, thus clearly describing the structural change in a PTP substrate trapping mutant (relative to the wildtype PTP sequence) that results in an alteration of function, that is, a change in catalytic properties of the PTP.

Thus, the specification describes in particular detail numerous species of PTP substrate trapping mutants. The specification describes the PTP catalytic domain, a highly conserved polypeptide region of approximately 250 amino acids that is found in a large number of PTPs, *i.e.*, not only in PTP1B (*e.g.*, page 1, line 25 through page 2, line 8, and publications cited therein). The specification also discloses that within the PTP catalytic domain is a signature active site motif [I/V]HCXAGXXR[S/T]G (SEQ ID NO:1) (*e.g.*, page 15, lines 13-14), which clearly contains a cysteine (C) residue and which can be identified in any PTP sequence using art-accepted sequence alignment techniques, as described, *e.g.*, at page 16, line 10 through page 17, line 27. The specification further teaches that PTPs contain twenty-seven invariant amino acid residues (*e.g.*, page 16, lines 10-14, and publications cited therein), among which is an aspartate residue present in the catalytic domain. In one embodiment of the invention, the invariant aspartate residue at position 181 in PTP1B is substituted with alanine; in another embodiment, the invariant aspartic acid at position 199 in PTP-PEST is mutated to alanine (*e.g.*, page 18, lines 9-12; page 44, Example 1). (On this point, Applicants respectfully note that in the Action, at page 3, line 9, the aspartic acid to alanine mutation at position 199 is incorrectly attributed to a mutant PTP1B. In fact, this D199A is a substitution in a distinct substrate trapping mutant of the PTP known as PTP-PEST, and is not a PTP1B mutation.) The specification also teaches that in certain embodiments of the invention, a PTPH1 substrate trapping mutant has the invariant aspartate at position 811 replaced, also with alanine (*e.g.*, page 21, lines 3-5). In addition, the specification provides the position of the invariant aspartate in the catalytic domains of several other PTPs (Figure 1D) from which a skilled artisan can readily

identify which aspartate residue to substitute in order to obtain a substrate trapping mutant of any aligned PTP sequence.

The specification further describes a substrate trapping mutant PTP derived from a PTP via substitution of the cysteine residue located in the phosphatase active site motif (SEQ ID NO:1) (*e.g.*, page 2, line 26 through page 3, line 19; page 15, lines 20-26). For example, a substrate trapping mutant of PTP1B has the cysteine residue at position 215 substituted with serine (*e.g.*, page 44, Example 1); and in another example, the cysteine at position 231 in PTP-PEST is substituted with a serine residue (*e.g.*, page 18, line 29 through page 19, line2; page 44, Example 1). The specification also provides an alignment of the signature motifs in numerous other PTPs from which a person skilled in the art can readily identify the invariant cysteine that may be substituted to obtain a substrate trapping mutant (*e.g.*, Figure 1D; *see also, e.g.*, page 15, lines 12-19).

A PTP substrate trapping mutant may further comprise a mutation in which a tyrosine residue is replaced with another amino acid, preferably a phenylalanine residue or another amino acid that is not capable of being phosphorylated (*see, e.g.*, specification, page 19, line 26 through page 20, line 12). Preferably, the tyrosine residue that is replaced is located in the PTP catalytic domain (*see, e.g.*, specification, page 20, lines 13-27). In a particular embodiment of the invention the tyrosine at position 676 in PTPH1 is replaced with phenylalanine, and in another embodiment the tyrosine at position 46 in PTP1B is substituted.

In view of the above remarks, Applicants respectfully submit that the specification more than adequately provides a detailed description of the relevant and identifying characteristics, structural as well as functional, of a representative number of PTP substrate trapping mutants that define the recited genus. Accordingly, Applicants submit that the present application describes <u>structural</u> characteristics of PTP substrate trapping mutants that correlate with functional characteristics, *i.e.*, reduced catalytic activity (Kcat) without a significant alteration of the Michaelis-Menten constant (Km) (*e.g.*, page 15, line 20 through page 16, line 16).

Similarly with regard to PTP substrates, Applicants submit that the specification provides sufficiently detailed, relevant and identifying characteristics of this genus for use in the claimed method. The specification describes a PTP substrate as any naturally or non-naturally

tyrosine-phosphorylated peptide, polypeptide, or protein that can specifically bind to and/or be dephosphorylated by a PTP (*e.g.*, page 23, lines 5-7). The specification also extensively describes selection of PTP substrates, for example, at pages 23-25 and at page 37, lines 6-24, including cited publications found therein. For instance, non-limiting examples of known PTP substrates include the proteins VCP, $p130^{cas}$, EGF receptor, p210 bcr:abl, MAP kinase, Shc, insulin receptor, lck (lymphocyte specific protein tyrosine kinase), and T cell receptor zeta chain (*e.g.*, page 23, lines 7-12, and publications cited therein).

Applicants submit that the specification also explicitly describes the structural and functional properties of tyrosine phosphorylated peptides that may be used as PTP substrates in the claimed method. The specification describes a PTP substrate as including a tyrosine phosphorylated peptide that may comprise a partial amino acid sequence, portion, region, fragment, variant, derivative, or the like from a naturally or non-naturally tyrosine-phosphorylated peptide, polypeptide, or protein that can specifically bind to and/or be dephosphorylated by a PTP (*e.g.*, page 23, lines 20-28). Examples of phosphorylated substrate peptides that are provided in the specification include F-crp-P (see Figs. 2, 3) that has the amino acid sequence (*i.e.*, a <u>structural</u> description), D-A-D-E-pY-L-NH_2 (SEQ ID NO:38), corresponding to residues 988-993 of the human EGF receptor (EGF-R) (*e.g.*, page 45, lines 5-18). The kinetic properties of this substrate (*i.e.*, function) are similar to the properties of the full-length EGF-R polypeptide: Km of ~3 μM and a Kcat of 72 s^{-1} (*e.g.*, page 45, lines 5-11, and references cited therein). A second EGF-R-derived tyrosine phosphorylated substrate peptide described in the specification is derived from amino acids 1170-1176 (N-A-E-pY-L-R-V, SEQ ID NO:37). Another PTP substrate that is disclosed in the present specification and that may be incorporated into the claimed method includes a peptide referred to as F-IR-P (see Figs. 2, 3), based on residues 1142-1152 of the human insulin receptor (*see, e.g.*, specification, page 46, lines 1-4). The peptide F-IR-P with only tyrosine 1146 phosphorylated (T-R-D-I-pY-E-T-D-Y-Y-R, SEQ ID NO:39) is a reported substrate for the PTPs LAR and CD45 and exhibits a Km of 27 μM and 34 μM for each PTP, respectively (*e.g.*, page 46, lines 1-9, and references cited therein). An additional peptide taught by the instant specification is F-lck-P (Figs. 2, 3), which corresponds to residues 500-509 (A-T-E-G-Q-pY-Q-P-Q-P, SEQ ID NO:40) of p56lck, the src-like lymphocyte specific protein tyrosine kinase that is a physiological substrate for CD45 and

that exhibits a Km of 130 μM (*e.g.*, page 46, lines 10-16). Thus, Applicants submit that the specification provides a detailed description of the relevant and identifying structural and functional characteristics of a representative number of PTP tyrosine phosphorylated substrates that define the recited genus for use in the claimed method.

Applicants therefore respectfully submit that in view of the foregoing remarks, the present specification adequately describes and reasonably conveys to a person skilled in the art that Applicants possessed a representative number of PTPs and PTP substrates for use in the claimed method at the time the Application was filed. Applicants therefore submit that the specification satisfies the written description requirement under 35 U.S.C. § 112, first paragraph, and respectfully request that the rejection of the claims be withdrawn.

REJECTION UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, ENABLEMENT

The PTO rejects claims 1-13 and 15-16 under 35 U.S.C. § 112, first paragraph, alleging that the specification does not enable a person skilled in the art to make and use the claimed invention. The PTO concedes that Applicants have enabled a method of identifying agents that alter the interaction of a PTP-1B-C215S, D181A or D199A (*sic*) and a labeled specific substrate, but asserts that the scope of the claims is not commensurate with the subject matter enabled by the disclosure. More specifically, the PTO alleges that undue experimentation would be required to practice the claimed invention, where a person skilled in the art would lack guidance from the specification as to which specific amino acid changes would result in a substrate trapping mutant PTP, and as to a substrate for such a mutant.

Applicants respectfully traverse this rejection and submit that as disclosed in the specification and recited in the instant claims, Applicants fully enabled the claimed invention at the time the instant application was filed. Contrary to the assertion by the PTO, Applicants submit that the instant specification provides abundant and explicit guidance regarding which amino acid changes in a PTP yield a substrate trapping mutant PTP, as discussed in detail above. Applicants also submit that as D199A is actually a substrate trapping PTP-PEST mutation and not a PTP-1B mutation (see note, *supra*), the PTO apparently concedes enablement of two species within the genus, namely, a substrate trapping mutant PTP1B (C215S, D181A) and a substrate trapping mutant PTP-PEST (D199A).

For reasons also discussed above, the specification clearly teaches how to obtain a suitable PTP substrate for use according to the invention and provides a significant number of representative examples (*e.g.*, EGF-R and EGF-R-derived peptides, insulin receptor and derived peptides, p56lck, VCP, p130^{cas}, p210 bcr:abl, MAP kinase, Shc, T cell receptor zeta chain, etc.; see specification at page 23, lines 5-12). Briefly, the specification teaches that substrate trapping mutant PTPs include those in which an invariant aspartate residue is replaced with an amino acid that does not cause significant alteration of the Km of the enzyme but that results in a reduction in Kcat to less than 1 per minute (less than 1 min^{-1}) (*e.g.*, page 18, lines 13-17; *see also* U.S. Pat. Nos. 5,912,138 and 5,951,979; U.S.A.N. 09/323,426; all incorporated by reference in the present specification). A substrate trapping PTP may further comprise other mutations, such as replacement of a catalytic domain cysteine residue, and/or replacement of at least one tyrosine residue with an amino acid that is not capable of being phosphorylated (*e.g.*, page 20, lines 13-27).

As also discussed above, the specification describes the highly conserved PTP catalytic domain containing a PTP signature active site motif (SEQ ID NO:1), and how to align PTP polypeptide sequences to identify the catalytic domain invariant aspartate residue which is replaced in substrate trapping mutant PTPs. The specification also describes, for example, representative species of substrate trapping mutant PTPs such as mutant PTP1B (*e.g.*, PTP1B-D181A, PTP1B-C215S, PTP1B-D181A/Y46F), mutant PTP-PEST (*e.g.*, PTP-PEST-D199A, PTP-PEST-C231S), mutant PTPH1 (*e.g.*, PTPH1-D811A, PTPH1-Y676F/D811A)(*e.g.*, page 15, lines 20 through page 23, line 2; U.S. Pat. Nos. 5,912,138 and 5,951,979; U.S.A.N. 09/323,426) and other substrate trapping mutant PTPs. Multiple exemplary PTP substrates, and methods for identifying such substrates using a substrate trapping mutant PTP, are also disclosed in the specification, as elaborated upon above. Assays to detect binding of a substrate trapping mutant PTP to a PTP substrate and to detect catalytic activity of the substrate trapping mutant PTP are also described in detail in the specification (*e.g.*, pages 27-32 and 32-38, respectively).

Accordingly, the specification more than amply teaches sequences of PTPs and relevant PTP substrates, including a significant number of representative examples of such PTP-PTP substrate combinations. The specification also clearly teaches how to identify which positions to substitute within PTP sequences to produce substrate trapping mutant PTPs having

the recited attributes, and how to engineer such substitutions, as well as methods for determining binding of a substrate to a mutant PTP.

Applicants therefore submit that the application provides a great deal of guidance, and that both the state of the art and the predictability of the art are high where a substantial number of PTPs and their cognate PTP substrates are known and disclosed in the application. The level of skill in the art is also known to be high, such that Applicants submit it would be highly reasonable for a person skilled in the art, given the present application, to select any PTP sequence and therefrom identify a substrate trapping mutant PTP having the recited properties which forms a complex with a suitable tyrosine phosphorylated peptide that is a PTP substrate, for use in the claimed invention method. In other words, the specification clearly discloses, and the amended claims expressly recite, that a substrate trapping mutant PTP comprises either substituted invariant aspartate or mutated catalytic domain cysteine, and that such mutant PTP forms a complex with its tyrosine phosphorylated PTP substrate peptide. Applicants therefore respectfully disagree with the position asserted by the PTO, that a large number of enzyme mutants and substrates would have to be screened because the effect of a mutation on a PTP is unpredictable *vis à vis* substrate trapping behavior. On the contrary, Applicants respectfully submit that undue experimentation would not be required to determine whether a particular mutant PTP-substrate combination forms a complex, where such determination must be regarded merely as a matter of permissible routine screening. (*In re Wands*, 858 F.2d 731, 736, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988) ("Enablement is not precluded by the necessity for some experimentation such as routine screening.").

Accordingly, Applicants submit that the present specification fully enables the skilled artisan to make and use the claimed invention without undue experimentation, and that the disclosure of the specification is commensurate with the scope of the claimed subject matter. Applicants respectfully submit that the present application satisfies all the requirements of 35 U.S.C. § 112, first paragraph, and therefore request that the rejection be withdrawn.

REJECTION UNDER 35 U.S.C. § 102

Claims 1-7 stand rejected under 35 U.S.C. §§ 102(b) and (e) for alleged lack of novelty. In particular, the PTO asserts that Tonks *et al.* (WO 98/04712) (§ 102(b)) and Tonks *et*

al. (U.S. Patent No. 5,912,138) (§ 102(e)) each teach a method for identifying an agent that alters the interaction between a substrate trapping mutant PTP and a substrate capable of generating a fluorescence signal. The PTO further alleges that each cited document discloses all elements recited in the dependent claims.

Applicants respectfully traverse this rejection and submit that each of the cited documents fails to anticipate the instant claims. Cancellation of claims 2, 6 and 7 by amendment herewith renders moot the rejections of these claims. Applicants respectfully submit that Tonks *et al.* (WO 98/04712) and Tonks *et al.* (U.S. Patent No. 5,912,138) fail to anticipate each and every limitation of the instant claims; therefore, the documents cannot be regarded as novelty destroying. Each document fails to teach or suggest a method for identifying an agent which alters the interaction between a PTP and a tyrosine phosphorylated polypeptide which is a substrate of the PTP, which method comprises, in pertinent part, contacting a substrate trapping mutant PTP and a labeled PTP substrate in the absence and presence of a candidate agent; and comparing a fluorescence energy signal that is a <u>fluorescence polarization</u> signal in the absence of the agent to a fluorescence energy signal that is a <u>fluorescence polarization</u> signal in the presence of the agent.

As disclosed in the present specification, fluorescence polarization permits solution-phase measurement of the fluorescence anisotrophy in polarized light of a free fluorescently labeled PTP substrate polypeptide, and of the labeled PTP substrate when it is involved in complex formation with a PTP (*e.g.*, page 29, line 18 through page 31, line 6). The difference in the polarization value of free detectably labeled PTP substrate polypeptide compared to the polarization value of a substrate trapping PTP mutant:substrate complex may be used to determine the ratio of complexed (*e.g.*, bound) substrate to free substrate (*see, e.g.*, specification, page 30, lines 20-27) without any requirement that complexed and free substrate be physically separated, or that a solid-phase component be employed (*e.g.*, page 13, line 16 through page 14, line 11; page 28, line 23 through page 30, line 27). This difference can be measured in the claimed method in the presence and absence of a candidate agent to determine whether the agent alters the interaction between a PTP and a PTP substrate.

By contrast, the cited documents teach that a phosphorylated protein/PTP complex may be detected by labeling the PTP substrate with any one of various detectable

moieties including enzymes, fluorescent materials, luminescent materials, and radioactive materials, followed by measuring the labeled PTP phosphorylated substrate only if it is present in the complex (see Tonks *et al.*, U.S. Patent No. 5,912,138, column 7, lines 7-27; Tonks *et al.*, WO 98/04712, page 12-13). The cited documents do not expressly teach, or in any way suggest, detection of a labeled PTP substrate using fluorescence polarization as specifically claimed by Applicants. Moreover, because the detection scheme disclosed and contemplated in each of the cited documents requires the physical separation of PTP-substrate complexes from unbound substrate (*e.g.*, U.S. 5,912,138, Col. 7, lines 7-27), each of these documents clearly does not specifically describe the use of fluorescence polarization which, as noted above, imposes no such requirement for separation.

Applicants submit that in view of the above remarks and the amendment introduced herewith, the subject matter of the claims is novel and satisfies the requirements of 35 U.S.C. § 102. Applicants therefore respectfully request that the rejections be withdrawn.

REJECTION UNDER 35 U.S.C. § 103

The PTO rejects claims 1, 6, and 8-16 under 35 U.S.C. § 103 for alleged obviousness over Tonks *et al.* (WO 98/04712) in view of Jia *et al.* (*Science* 268:1754-58 (1995)). The PTO alleges that a person having ordinary skill in the art would have found it obvious to arrive at the claimed invention by combining the teachings of Tonks *et al.* with the teachings of Jia *et al.*

Applicants traverse this rejection and submit that the documents cited by the PTO, alone or in combination, fail to teach or suggest the subject matter of the instant claims. Cancellation of claims 2, 6 and 7 by amendment herewith renders moot the rejections of these claims. Applicants respectfully submit that the PTO has not established a *prima facie* case of obviousness. (*See In re Mayne*, 104 F.3d 133, 1341-43, 41 U.S.P.Q.2d 1451 (Fed. Cir. 1997) (PTO has the burden of showing a *prima facie* case of obviousness.)). The Court in *Mayne* required that the PTO show (1) that the combined references teach or suggest all claim limitations; (2) that the references provide some teaching, suggestion, or motivation to combine or modify the teachings of the prior art to produce the claimed invention; and (3) that the combined teachings of the references indicate that by combining the references, a person having

ordinary skill in the art will achieve the claimed invention with a reasonable expectation of success. When rejection of claims depends upon a combination of prior art references, a teaching, motivation, or suggestion to combine the references must exist and be proven by the PTO. (*See In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453 (Fed. Cir. 1998)).

Applicants respectfully submit that Tonks *et al.* and Jia *et al.* either alone or in combination, fail to teach or suggest the presently claimed invention. These documents would not have motivated a person having ordinary skill in the art to obtain Applicants' claimed invention with a reasonable expectation of success. As discussed above with respect to novelty of the subject invention, Tonks *et al.* fail to teach or suggest the fluorescence energy signal as being a fluorescence polarization signal. Jia *et al.* are also silent with regard to detecting a fluorescence polarization signal in a method comprising contacting a substrate trapping mutant PTP and a PTP substrate in the absence and presence of a candidate agent. Applicants submit that the documents alone or in combination as cited by the PTO fall short of anticipating the present invention.

Also, as conceded by the PTO (see Office Action, page 8, third full paragraph), "Tonks *et al.* does not teach a human PTP-1B wherein Tyr-46 is substituted with a phenylalanine residue." Applicants submit that Jia *et al.* fail to teach what Tonks *et al.* do not. The combination of Jia *et al.* with Tonks *et al.* does not suggest <u>every</u> element of the presently claimed invention. Jia *et al.* fail to teach *any* method for identifying an agent that alters the interaction between a PTP and a PTP substrate by using a substrate trapping mutant PTP. Jia *et al.* also fail to teach or suggest any substrate trapping mutant PTP, and also fail to teach or suggest substitution of any residue in the catalytic domain of PTP1B to obtain a PTP1B substrate trapping mutant. Applicants submit therefore that Tonks *et al.* in view of Jia *et al.* would not have motivated an ordinarily skilled artisan to arrive at the claimed invention. The PTO fails to point to any disclosure in either document suggesting the desirability of making such combination of teachings.

Contrary to the assertion by the PTO, Applicants submit that the disclosure in Jia *et al.* that a *Yersinia* PTP has a phenylalanine residue at a comparable position to the tyrosine at position 46 in PTP1B would not have motivated a person having ordinary skill in the art to combine this teaching with Tonks *et al.* to obtain a PTP1B substrate trapping mutant, with any

reasonable expectation of success. Jia *et al.* merely point out that the tyrosine residue at position 46 of PTP1B is one of several nonpolar residues that form the recognition site for the phenyl ring of pTyr in a tyrosine phosphorylated substrate (Jia *et al.*, page 1755, first column). Applicants therefore submit that, if anything, this observation by Jia *et al.* teaches away from substituting tyrosine at position 46 of PTP1B to obtain a substrate trapping mutant. Briefly, because the catalytically active wildtype *Yersinia* PTP features a phenylalanine residue at the position comparable to position 46 (tyrosine) in PTP1B, a person having ordinary skill in the art would reasonably expect that substitution of the tyrosine residue in PTP1B with phenylalanine would *not* affect catalytic activity and thus would not provide a PTP1B substrate trapping mutant with attenuated catalytic activity.

Applicants respectfully submit that for the reasons discussed above, a *prima facie* case of obviousness has not been established, and that the claimed invention is nonobvious as required by 35 U.S.C. § 103. Applicants therefore respectfully request that the rejection of the claims be withdrawn.

Applicants respectfully submit that all claims remaining in the application are now allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,
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Enclosures:
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Version with Markings to Show Changes Made

In the Specification:

The paragraph beginning at page 2, line 26, has been has been amended as follows:

(Twice Amended) The PTP family of enzymes contains a common evolutionarily conserved segment of approximately 250 amino acids known as the PTP catalytic domain. Within this conserved domain is a unique signature sequence motif,

[I/V]HCXAGXXR[S/T)G SEQ ID NO: ~~36~~1,

that is invariant among all PTPs. The cysteine residue in this motif is invariant in members of the family and is known to be essential for catalysis of the phosphotyrosine dephosphorylation reaction. It functions as a nucleophile to attack the phosphate moiety present on a phosphotyrosine residue of the incoming substrate. If the cysteine residue is altered by site-directed mutagenesis to serine (*e.g.*, in cysteine-to-serine or "CS" mutants) or alanine (*e.g.*, cysteine-to-alanine or "CA" mutants), the resulting PTP is catalytically deficient but retains the ability to complex with, or bind, its substrate, at least *in vitro*.

The paragraph beginning at page 11, line 16, has been has been amended as follows:

Figure 1 shows a multiple amino acid sequence alignment of the catalytic domains of various PTPs. The positions of amino acid residues of PTP1B that interact with substrate are indicated with small arrowheads, and the residue numbering at the bottom of the alignment corresponds to that for PTP1B. Figs. 1A-1E show a multiple sequence alignment of the catalytic domains of PTPs (SEQ ID NOS:~~1~~2-~~35~~36). Cytosolic eukaryotic PTPs and domain 1 of RPTPs are combined into one group; domains 2 of RPTPs are in a second group and the

Yersinia PTP is in a third. Invariant residues shared among all three groups are shown in lower case. Invariant and highly conserved residues within a group are shown in italics and bold, respectively. Within the *Yersinia* PTP sequence, residues that are either invariant or highly conserved between the cytosolic and RPTP domain sequences are in italics and bold, respectively.

The paragraph beginning at page 15, line 13, has been amended as follows:

(Twice Amended) As defined herein, a phosphatase is a member of the PTP family if it contains the signature motif [I/V]HCXAGXXR[S/T]G (SEQ ID NO: ~~36~~1). Dual specificity PTPs, *i.e.*, PTPs which dephosphorylate both phosphorylated tyrosine and phosphorylated serine or threonine, are also suitable for use in the invention. Appropriate PTPs for use in the present invention may be any PTP family member including, but not limited to, PTP1B, PTP-PEST, PTPγ, MKP-1, DEP-1, PTPμ, PTPX1, PTPX10, SHP2, PTP-PEZ, PTP-MEG1, LC-PTP, TC-PTP, CD45, LAR and PTPH1, and mutated forms thereof.

The paragraph beginning at page 16, line 4 and ending at page 16, line 24, has been amended as follows.

(Twice Amended) As noted above, substrate trapping mutant PTPs are derived from wildtype PTPs that have been mutated such that the wildtype protein tyrosine phosphatase catalytic domain invariant aspartate residue is replaced with an amino acid which does not cause significant alteration of the Km of the enzyme but which results in a reduction in Kcat to less than 1 per minute. Optionally, a catalytic domain cysteine residue is also replaced with a different amino acid, and/or at least one wildtype tyrosine residue is replaced with an amino acid that is not capable of being phosphorylated. In this regard, amino acid sequence analysis of known PTPs reveals the presence of ~~twenty~~ twenty-seven invariant residues within the PTP primary structure (Barford *et al.*, 1994 *Science 263*:1397-1404; Jia *et al.*, 1995 *Science 268*:1754-1758), including an aspartate residue in the catalytic domain that is invariant among PTP family members. When the amino acid sequences of multiple PTP family members are

aligned (see, for instance, Figure 1A-E in U.S.A.N. 09/334,575; see also, *e.g.*, Barford *et al.*, 1995 *Nature Struct. Biol.* 2:1043), this invariant aspartate residue may be readily identified in the catalytic domain region of each PTP sequence at a corresponding position relative to the PTP signature sequence motif [I/V]HCXAGXXR[S/T]G (SEQ ID NO:~~2~~1), which is invariant among all PTPs (see, *e.g.*, WO98/04712; Flint *et al.*, 1997 *Proc. Nat. Acad. Sci.* 94:1680 and references cited therein). However, the exact amino acid sequence position numbers of catalytic domain invariant aspartate residues may be different from one PTP to another, due to sequence shifts that may be imposed to maximize alignment of the various PTP sequences (see, *e.g.*, Barford *et al.*, 1995 *Nature Struct. Biol.* 2:1043 for an alignment of various PTP sequences).

In the Claims:

Claims 2, 6 and 7 have been cancelled.

Claims 1 and 8 have been amended as follows:

1. (Amended) A method for identifying an agent which alters the interaction between a protein tyrosine phosphatase and a tyrosine phosphorylated polypeptide which is a substrate of the protein tyrosine phosphatase, comprising:

(a) contacting in the absence and in the presence of a candidate agent, a substrate trapping mutant of a protein tyrosine phosphatase and a detectably labeled tyrosine phosphorylated peptide which is a substrate of the protein tyrosine phosphatase under conditions and for a time sufficient to permit formation of a complex between the tyrosine phosphorylated peptide and the substrate trapping mutant protein tyrosine phosphatase, wherein the substrate is capable of generating a fluorescence energy signal and wherein the substrate trapping mutant protein tyrosine phosphatase is selected from the group consisting of

(i) a protein tyrosine phosphatase in which wildtype protein tyrosine phosphatase catalytic domain invariant aspartate residue is replaced with an amino acid which does not cause significant alteration of the Km of the enzyme but which results in a reduction in Kcat to less than 1 per minute, and

(ii) a protein tyrosine phosphatase in which a cysteine that is present in a signature sequence motif as set forth in SEQ ID NO:1 within a wildtype protein tyrosine phosphatase catalytic domain is mutated at an amino acid position occupied by a cysteine residue; and

(b) comparing the fluorescence energy signal level in the absence of the agent to the fluorescence energy signal level in the presence of the agent, wherein a difference in the fluorescence energy signal level indicates the agent alters formation of a complex between the protein tyrosine phosphatase and the substrate, and wherein the fluorescence energy signal is a fluorescence polarization signal.

2. (Canceled) The method of claim 1 wherein the fluorescence energy signal is a fluorescent polarization signal.

3. The method of claim 1 wherein the detectably labeled tyrosine phosphorylated peptide comprises a fluorophore.

4. The method of claim 3 wherein the fluorophore is selected from the group consisting of fluorescein, rhodamine, Texas Red, AlexaFluor-594, AlexaFluor-488, Oregon Green, BODIPY-FL and Cy-5.

5. The method of claim 1 wherein the substrate comprises a polypeptide sequence derived from a protein selected from the group consisting of VCP, $p130^{cas}$, EGF receptor, p210 bcr:abl, MAP kinase, Shc, insulin receptor, lck and T cell receptor zeta chain.

6. (Canceled) The method of claim 1 wherein the substrate trapping mutant protein tyrosine phosphatase comprises a protein tyrosine phosphatase in which the wildtype protein tyrosine phosphatase catalytic domain invariant aspartate residue is replaced with an amino acid which does not cause significant alteration of the Km of the enzyme but which results in a reduction in Kcat to less than 1 per minute.

7. (Canceled) The method of claim 1 wherein the substrate trapping mutant protein tyrosine phosphatase comprises a protein tyrosine phosphatase in which the wildtype protein tyrosine phosphatase catalytic domain is mutated at an amino acid position occupied by a cysteine residue.

8. (Amended) The method of claim 16 wherein the substrate trapping mutant protein tyrosine phosphatase comprises a protein tyrosine phosphatase in which at least one wildtype tyrosine residue is replaced with an amino acid that is not capable of being phosphorylated.

9. The method of claim 8 wherein at least one wildtype tyrosine residue is replaced with an amino acid selected from the group consisting of alanine, cysteine, aspartic acid, glutamine, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, arginine, valine and tryptophan.

10. The method of claim 8 wherein at least one tyrosine residue that is located in a protein tyrosine phosphatase catalytic domain is replaced.

11. The method of claim 8 wherein at least one tyrosine residue that is located in a protein tyrosine phosphatase active site is replaced.

12. The method of claim 8 wherein the wildtype tyrosine residue is replaced with phenylalanine.

13. The method of claim 8 wherein the wildtype tyrosine residue that is replaced is a protein tyrosine phosphatase conserved residue.

14. (Withdrawn) The method of claim 13 wherein the conserved residue corresponds to tyrosine at amino acid position 676 in human PTPH1.

15. The method of claim 8 wherein at least one tyrosine residue is replaced with an amino acid that stabilizes a complex comprising the protein tyrosine phosphatase and at least one substrate molecule.

16. The method of claim 8 wherein the substrate trapping mutant protein tyrosine phosphatase is a mutated protein tyrosine phosphatase selected from the group consisting of PTP1B, PTP-PEST, PTPγ, MKP-1, DEP-1, PTPμ, PTPX1, PTPX10, SHP2, PTP-PEZ, PTP-MEG1, LC-PTP, TC-PTP, CD45, LAR and PTPH1.

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